PURIFICATION OF A COLONY STIMULATING FACTOR FROM CULTURE CELL LINES PROPAGATED FROM HUMAN LUNG

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1. Introduction

Colony stimulating factor (CSF) is required for the in vitro growth of marrow myeloid colonies (CFU-C) and is thought to play an important role in the regulation of granulopoiesis in vivo [1–3]. Although CSF has been isolated from multiple murine and human tissues [4–6], the sources of human CSF have been extremely limited. Human urinary CSF has been purified to homogeneity but curiously this glycoprotein stimulates CFU-C growth in murine, but not in human bone marrow [7]. Recently human CSF from the conditioned medium of human placenta and human lung have been partially purified [8,9]. However, major contaminants derived from tissue and serum presented considerable difficulty in purification. To overcome this problem we have attempted and succeeded in propagating cell lines from autopsy lung tissue which secrete CSF into the medium. Serum-free conditioned medium prepared from these cultures provides an excellent source of CSF.

2. Materials and methods

Dulbecco's modified Eagle's medium (DME), fetal calf serum, horse serum, were obtained from Grand Island Biological Company, NY. Ultrogel (AcA44, Ampholine, Ultrodex) and preparative flat bed gel isoelectrofocusing apparatus were products of LKB, Rockville, MD. All chemicals were reagent grade.

C57B/6J mice used as marrow donors for CSF assay were from Jackson Laboratories, Bar Harbour, ME. Human marrow was obtained from normal volunteers.

2.1. *Cell cultures*

Lung tissue was collected under sterile conditions 8–16 h postmortem, and placed in 150 × 25 mm Falcon plastic culture plates. It was minced with sterile scissors, distributed among several plates and dispersed in DME with 10% fetal calf serum and 2.5% horse serum, 5 ml/plate. The plates were placed in a humidified incubator in an atmosphere of 10% CO_2 in air. Transfers were accomplished by trypsinizing the cells and distributing them into plates each containing 10 ml medium.

2.2. *Preparation of CSF-rich serum-free conditioned medium*

When cultures have grown to near confluence, the medium was aspirated and the plates rinsed twice with 5 ml Hank's balanced salt solution and 3 ml serum free DME added to each plate. Incubation was then resumed for an additional 48 h after which the medium was harvested.

2.3. *Assay for CSF activity*

This was carried out by the method in [1] as described [10]. Mouse marrow cells were used for routine assays. However, the pooled fractions from every purification step was assayed in both mouse and human marrow. A unit of activity is defined as the amount of CSF which stimulates the formation of one colony under the specified assay condition.

3. Results

3.1. *Cell cultures*

After 6–10 days many colonies were observed, some of which were composed of epithelial like cells, others fibroblastic in appearance. After several transfers there was rapid loss of epithelial colonies and replacement by fibroblastic growth with few scattered 'round' cells. By the 5th transfer the cultures became completely fibroblastic in appearance, as shown in fig.1.

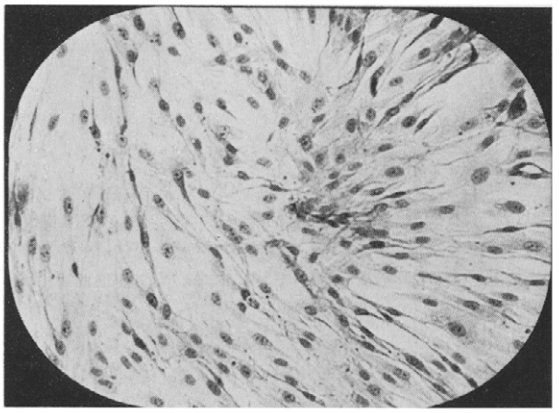

Fig.1. Morphology of human lung cell cultures × 75.

3.2. *Colony stimulating factor in conditioned medium*

Conditioned medium obtained from 6–8 day cultures stimulates the growth of granulocytic and macrophagic colonies in both mouse and human bone marrow, in each case yielding a dose versus response pattern shown in fig.2a, b.

Conditioned serum-free medium prepared from these cultures as described under section 2 had spec. act. 15 000–25 000 units/mg protein. Cell lines have undergone 15–20 transfers without decline in CSF production. Thereafter the cultures often showed rapid degeneration.

3.3. *Purification of cultured-lung CSF*

Purification was carried out by a two step procedure involving isoelectrofocusing and filtration in Ultrogel. A summary of the results is shown in table 1.

3.4. *Isoelectrofocusing*

CSF-rich serum-free conditioned medium, 900 ml, was concentrated to 3 ml by Amicon ultrafiltration using a PM 10 membrane and then dialyzed against distilled water overnight. The sample was then subjected to preparative flat bed isoelectrofocusing in granulated gel as described in LKB Application Note No. 198. The sample was applied to the Ultrodex gel bed as a narrow zone and isoelectrofocusing was conducted overnight. Each fraction was eluted with 5 ml 0.1 M NaCl containing 0.1% Tween 20. Eluates were dialyzed against distilled water, sterilized by

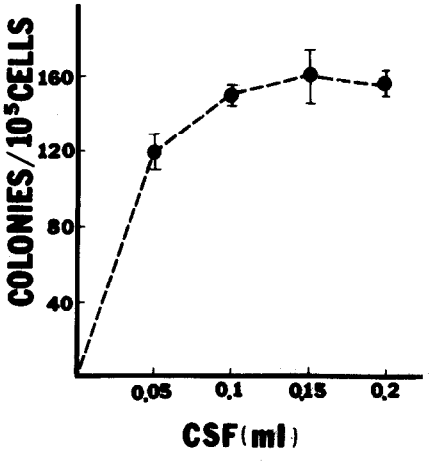

Fig.2A

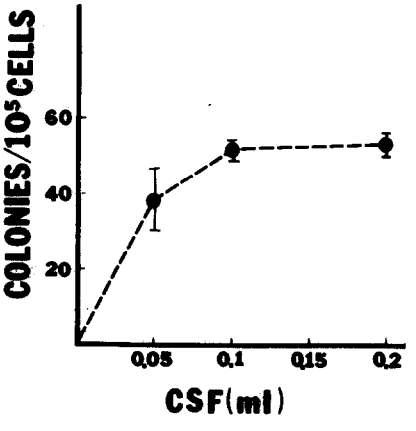

Fig.2B

Fig.2. Stimulation of mouse (A) and human (B) marrow CFU-C growth by CSF in serum-free conditioned medium prepared from human lung cell culture.

Table 1
Purification of CSF from serum-free conditioned medium of cultured lung cells

	Purification	Source of marrow cells for assay	Total protein (mg)	Total activity (units)	Specific activity (unit/mg)	Purification (fold)	Recovery
1.	Conditioned medium	Mouse	17.7	2.95×10^5	1.67×10^4	1	100
		Human		1.23×10^5	0.70×10^4	1	100
2.	Preparative iso-electrofocusing	Mouse	0.354	1.77×10^5	5.0×10^5	30	61
		Human		1.06×10^5	3.0×10^5	43	86
3.	Gel filtration	Mouse	0.002	0.36×10^5	1.8×10^7	1078	12.2
		Human		0.23×10^5	1.2×10^7	1710	18

filtration, and assayed for CSF activity using mouse marrow. The pH of each fraction was determined prior to dialysis and $A_{280\ nm}$ value after dialysis. Protein concentration was measured according to [11]. The elution profile is shown in fig.3. A 30-fold purification was achieved by this step with 61% recovery.

3.5. *Filtration in ultrogel*

The fractions with highest specific activity (fractions 1–5) from the previous step were pooled, concentrated and 1 ml sample was loaded on a 1.2 X 100 cm Ultrogel AcA44 column equilibrated with 0.1 M NaCl containing 0.01% Tween 20, 20 units/ml penicillin, and 20 μg/ml streptomycin. Elution was carried out with the same buffer and 1 ml fractions were collected and their A_{280} and CSF activity were determined as described above using mouse marrow. The molecular weight of CSF was estimated by calibrating the column with standard markers including Blue Dextran (V_0), bovine serum albumin (mol. wt 68 000) myoglobin (mol. wt 17 000) and phenol red (V_R). The elution profile is shown in fig.4. Approximately 30-fold purification was achieved by this step yielding final spec. act. 1.8×10^7 units/mg. The estimated mol. wt was 40 000.

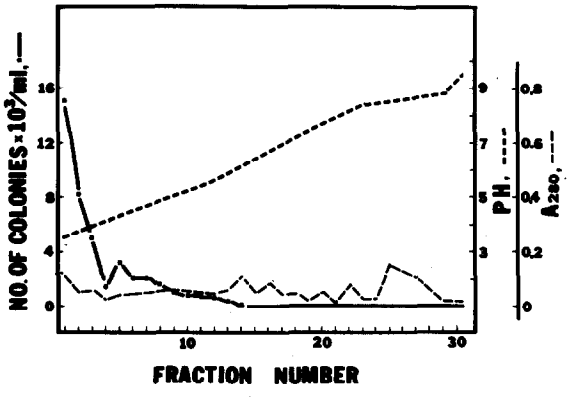

Fig.3. Preparative flat bed isoelectrofocusing of concentrated CSF-rich serum-free conditioned medium from human lung cell culture.

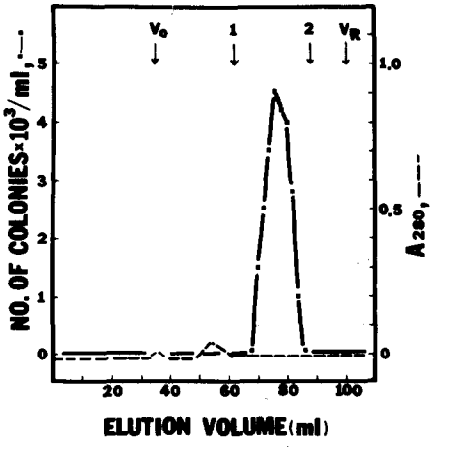

Fig.4. Gel filtration, on Ultrogel AcA44, of pooled active fractions from the isoelectrofocusing step shown in fig.3. (1) Bovine serum albumin; (2) myoglobin.

4. Discussion

Conditioned serum-free medium prepared from lung cell cultures is rich in CSF with a relatively high specific activity of 15 000–25 000 units/mg. Therefore, as a starting material for purification it provides a very important advantage over whole lung tissue-conditioned medium and placenta-conditioned medium both of which have specific activities in the range of only 400–1200 units/mg [9]. The ease of culturing lung tissue allowing the simultaneous cultivation of any number of cell lines which can be carried through 15–20 transfers promises to make this approach a practical one for obtaining highly purified human CSF. Attempts to cultivate CSF-producing 'fibroblastic' cultures from human autopsy lung have been virtually uniformly successful with only an occasional failure due to a contaminated tissue specimen. The two-step purification procedure described here, especially the preparative isoelectrofocusing, proved to be extremely effective and should prove valuable in large scale CSF purification.

The final spec. act. 1.8×10^7 units/mg obtained is the highest achieved for a CSF from a human source with the exception of human urinary CSF which has been purified to spec. act. 1.6×10^8 units/mg as assayed in mouse marrow [12]. However, in contrast to purified urinary CSF which stimulates colony growth only in mouse marrow, purified CSF from 'fibroblastic' lung cultures stimulates colony growth in both human and murine marrow. Human placental CSF has been purified to a specific activity of only 2.5×10^5 units/mg protein.

It is uncertain what cell type is secreting CSF in these cultures. Alveolar macrophages have been shown to produce CSF [13]. Electron microscopic examination of our cell cultures done after the 5th transfer showed only an occasional cell which could be identified as a macrophage. The fact that CSF production by these cultures showed no decline with repeated transfers and after all growth was apparently fibroblastic suggests that lung fibroblasts are the source of CSF, but further cytological and biochemical studies are necessary before a definite conclusion can be made. Also whether the CSF obtained from lung cell cultures is identical to that derived from tissue-conditioned medium, needs further study.

Acknowledgements

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